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Cognitive, Behavioral, and Neuroanatomical Assessment of Two Unrelated Male Children Expressing *FRAXE*

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Standardized cognitive, behavioral, and neuroanatomical data are presented on 2 unrelated boys with the *FRAXE* (*FMR2*) GCC expansion mutation. In the context of normal IQ, both boys had a history of developmental delay, including significant problems with communication, attention, and overactivity. Additionally, one child was diagnosed with autistic disorder. Data from these 2 cases are compared to analogous information from previous reports about individuals with the *FRAXE* or *FRAXA* (*FMR1*) mutation. These comparisons support the idea that *FRAXE* is associated with nonspecific developmental delay and possibly high-functioning autism. *Am. J. Med. Genet. 74: 73–81, 1997.*

KEY WORDS: nonspecific developmental delay; CGG expansion; autism; *FMR1; FMR2; FRAXA; FRAXE*

INTRODUCTION

Fragile X E (*FRAXE*) is a folate-sensitive, simple tandem-repeat mutation at Xq27.3 [Sutherland and Richards, 1995]. This mutation is associated with the downregulation of the distal *FMR2* gene, which is expressed in the adult and fetal brain as well as in other tissues [Gecz et al., 1996; Gu et al., 1996]. The *FRAXE* site is also located in close proximity to, and between, *FRAXA* and *FRAXF*. All three fragile sites are typically composed of hypermethylated CG-rich triplet expansions. *FRAXF* does not appear to be associated with any obvious phenotype [Parrish et al., 1994; Ritchie et al., 1994], indicating that such a mutation may have no clinical significance. However, *FRAXA* is well-known as a mutation which downregulates *FMR1* gene expression [Verkerk et al., 1991; Pieretti et al., 1991; Verheij et al., 1993] and leads to a cognitive and behavioral syndrome, including mental retardation and autistic-spectrum abnormalities [reviewed in Hagerman, 1991]. At present it is unclear if an analogous *FRAXE* neurobehavioral syndrome exists.

Some case reports are suggestive of an association between the hypermethylated *FRAXE* mutation and nonspecific, but clinically detectable, mental impairment [Knight et al., 1993, 1994; Hamel et al., 1994; Mulley et al., 1995]. These reports are limited in number and scope, so that very little standardized and comprehensive data have been presented about individuals with the *FRAXE* mutation. Among the 66 cases of *FRAXE* reported to date, standardized IQ scores were included for only 8 individuals across three separate families [Hamel et al., 1994; Mulley et al., 1995; Knight et al., 1996]. This group was comprised of 5 males and 3 females with full-scale IQs ranging from 53–104 and 49–73, respectively. The IQ subtest profiles and other cognitive measures reported in these studies were not suggestive of any specific cognitive profile (i.e., obvious strengths or weaknesses) in the affected subjects. However, 7 of the 8 individuals had full-scale IQs at or below the borderline range (i.e., <74). Thus, the *FRAXE* cognitive phenotype, if specific, has yet to be well-defined.

Even more limited than the cognitive description of the few *FRAXE* cases reported are the behavioral evaluations for these same individuals. Behavioral descriptions are primarily limited to information on educational placement or occupation. Of 36 male *FRAXE* cases thus far reported, behavioral problems have been

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described for only 5 individuals. These problems included poor adaptive skills, anxiety, aggressiveness, repetitive speech, and stuttering. It may be that such problems are rare in *FRAXE*; however, it is also possible that these problems were not directly evaluated, and thus overlooked, in the remaining cases.

In this report, comprehensive and longitudinal neurobehavioral data from 2 new unrelated male *FRAXE* cases are presented. More limited data are also presented about the biological mothers of each subject. These data will be compared to other previously described cases of *FRAXE*, and to what is currently know about the *FRAXA* phenotype. Included are unique neuroanatomical data on the males with *FRAXE*.

SUBJECTS AND METHODS

Ascertainment of Subjects

Because of their proximity, *FRAXE* and the *FMR1* mutation (*FRAXA*) cannot be distinguished from one another by standard cytogenetic screening [Sutherland and Baker, 1992]. Both young male probands described in this paper were originally misclassified as having *FRAXA* based on positive cytogenetic results [Lubs, 1969; Sutherland, 1977]. When DNA testing for *FRAXA* became available, it was discovered that these subjects did not have the typical expansion mutation associated with fragile X syndrome [Rousseau et al., 1991]. Subsequent direct DNA testing [Knight et al., 1993] demonstrated a GCC expansion at the *FRAXE* locus in these 2 subjects.

Subject 1 (Fig. 1, family 1, individual III-1) was referred by another team of specialists who evaluated him at age 1 year. These specialists reported that subject 1 had mild-to-moderate hypotonia and gross motor and speech delays. Concurrent cytogenetic testing identified the fragile X chromosome in subject 1 as well as in his mother and maternal grandmother. As a component of ongoing studies on fragile X syndrome, subject 1 received additional neurodevelopmental evaluations at age 1 year, 5 months, and age 4 years, 4 months.

Subject 2 (Fig. 1, family 2, individual II-2) was originally referred at age 4 years, 10 months for clinical evaluation because of developmental delay and possible autism. A multidisciplinary assessment led to the diagnosis of "autistic-like features" and communication disorder, characterized by expressive and receptive language delays despite age-appropriate naming skills. Cytogenetic studies from that evaluation period identified the fragile X chromosome. Follow-up evaluations of subject 2 were conducted at ages 8 years, 2 months, and 12 years, 3 months.

Cytogenetic and DNA Analysis

Standard cytogenetic techniques were used on lymphocyte-derived cells for detection of the fragile X chromosome. Lymphocyte-derived DNA was examined using previously described Southern blot [Rousseau et al., 1991] and PCR [Fu et al., 1991] assays to characterize the size and methylation status of the *FMR1* promoter region (*FRAXA* locus). To detect GCC amplifications across the *FRAXE* fragile site, and to test the methylation status of the associated CpG island, DNA samples were digested with *Hind*III and with *Hind*III + *Bss*HII, *Hind*III + *Sac*II, and *Hind*III + *Not*I, and then probed with OxE20, as described previously [Knight et al., 1993]. For the original *Hind*III analyses, DNA was derived from peripheral blood lymphocytes, whereas for methylation analyses, DNA was derived from lymphoblastoid cell lines.

Medical and Developmental History Review

Each subject's available medical records were reviewed, and for subject 2, school records were also reviewed. Additionally, the parents of each subject completed a standard medical and developmental history form including questions focusing on pre-, peri-, and postnatal events and developmental milestones.

Psychological Evaluations

Assessments were often consistent for both subjects; however, the individual test batteries did vary because of the age difference between the two probands, and also because the biological mother of subject 2 agreed to additional testing. For all assessments, total and/or factor scores were calculated and comparisons were made to normative or other published data.

Cognitive testing. Standardized instruments used to evaluate both subjects and the ages when testing took place are listed in Table I. Additional assessments were also used to further evaluate: 1) both subjects' expressive and receptive language, 2) subject 1's visual-motor skills, and 3) subject 2's cognitive ability (see Appendix A). During their initial visit, the biological mother of each subject was administered the Wechsler Adult Intelligence Scale (WAIS-R) [Wechsler, 1981], and several brief, standardized neuropsychological tests were used to assess specific areas of cognitive ability.

Behavioral assessments. Psychiatric status, adaptive skills, and autistic behaviors of each subject were assessed using semistructured parent interviews and questionnaires, including the Vineland Adaptive Behavior Scales [Sparrow et al., 1984] and the Diagnostic Interview for Childhood and Adolescence—Parent Version (DICA-P) [Reich and Welner, 1988]. Categorical diagnoses from these questionnaires were based on criteria from the Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, Revised (DSM-III-R). Problem behaviors addressed included, but were not limited to, autistic spectrum behaviors, irritability, attentional difficulties, anxiety, and social dysfunction.

Psychiatric and personality status of subject 2's mother was evaluated with the Schedule for Affective Disorders and Schizophrenia—Lifetime Version [Endicott and Spitzer, 1978], the NEO personality inventory [Costa and McCrae, 1985], and several other rater- or self-administered questionnaires.

Neuroanatomical Assessment

MRI brain scans of both male subjects were quantitatively assessed using methods previously applied to *FRAXA* and normal subjects [Reiss et al., 1991, 1995]. Measurements included midline areas of structures such as the cerebellum vermis and corpus callosum along with volumes of subcortical nuclei, cortical grey matter, white matter, and cerebrospinal fluid (CSF).

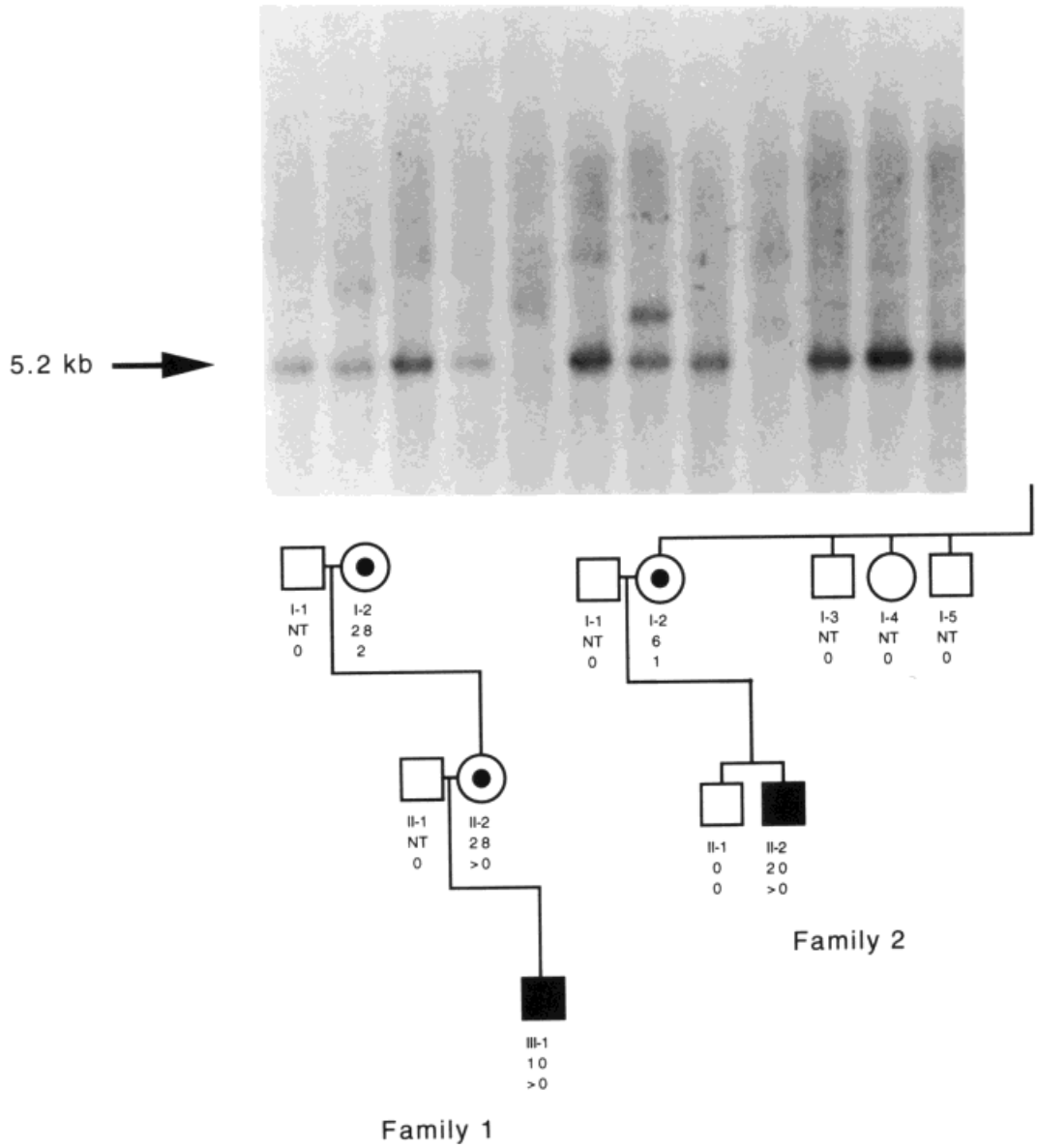

Fig. 1. *FRAXE* Southern blot. Family numbers correspond to the subject numbers. Subclone OXE20 was used as a hybridization probe against a Southern blot containing *Hind*III-digested DNA. A normal pattern yields a single band at 5.2 kb. Larger band sizes indicate the presence of the *FRAXE* GCC triplet repeat expansion mutation. Beneath each individual pedigree symbol are the ID number, percent fragile site expression, and size of the *FRAXE* expansion estimated in kb. NT, not tested; >0, a smear of heterogeneous *FRAXE* expansion fragments >5.2 kb; 0, a negative karyotype and/or normal *FRAXE* locus. All subjects tested had *FRAXA* allele sizes within normal range.

RESULTS
Cytogenetic and DNA Analyses

Subject 1's family. Subject 1's pedigree is shown in Figure 1, with a summary of each individual's genetic information. At age 1 year, 5 months, subject 1 (family 1, III-1) was found to have a fragile site at Xq 27.3 in 5/50 cells. Cytogenetic screening done elsewhere showed subject 1's biological mother and grandmother to have 14/50 and 8/28 fragile X chromosomes, respectively.

Southern blot analysis on subject 1's family detected a very small unmethylated *FMR1* expansion in the proband, his mother, and his maternal grandmother. For the proband, PCR analysis was used to confirm that this allele was in the high normal range at 50 CGG repeats [Fu et al., 1991] (data not shown).

Southern blot analysis also identified the *FRAXE* mutation in subject 1, his mother, and his maternal grandmother. Subject 1 had a "smear" of DNA >5.2 kb in his lymphocytes (Fig. 1, lane 5), and a discrete band ~8.0 kb in lymphoblastoid-derived cells (Fig. 2, lane 5), which are likely to be more genetically homogenous due to the clonal nature of such an immortal cell line. Additionally, the ~8.0-kb fragment was refractory to diges-

TABLE I. Summary of Cognitive and Behavior Assessments for Both *FRAXE* Males*

	Subject 1	Subject 1	Subject 2	Subject 2[b]	Subject 2
Age (years/months)	1/5	4/4	8/2	10/5	12/3
Bayley-Mental Scale	63				
Stanford-Binet Composite		88	88	98	
Quantitative		88	112	106	
Verbal		96	84	82	
Short-term memory		99	70	86	
Abstract visual		77	95	120	
WISC-R (FSIQ)					100
Verbal					85
Performance					117
Vineland Composite	N/C[a]	75	57	53	
Communication	88	87	67	69	
Daily living	95	84	57	44	
Socialization	105	84	63	61	52
Motor skills		68			
Autism or PDD diagnosis	No	No	Autism		Autism
Attention deficit hyperactivity disorder		Mild	Moderate		Mild-to-moderate

* Standard scores and DSM-III-R diagnoses are listed.

[a] Motor Skills Domain was not done, therefore, the composite score was not calculated.

[b] Results are from an educational evaluation.

tion by methyl-sensitive enzymes *Bss*HII, *Sac*II, and *Not*I (Fig. 2, lanes 6–8). Subject 1's mother had a "smear" of expansion fragments >5.2 kb and a normal 5.2 kb fragment (Fig. 1, lane 4). Methylation analysis of her expanded fragments with *Not*I was inconclusive because of the excessive heterogeneity of her *FRAXE* alleles (data not shown). Subject 1's maternal grandmother had an expanded fragment of ~7.2 kb in addition to a normal 5.2-kb fragment (Fig. 1, lane 2), and her expanded *FRAXE* fragment was completely refractory to *Not*I digestion (data not shown).

Subject 2's family. Subject 2's pedigree and genetic information are shown in Figure 1. At age 12 years, 3 months, subject 2 (family 2, II-2) was found to have fragile sites at Xq27.3 in 20/100 cells examined. Subject 2's biological mother had fragile X chromosomes in 6/100 cells. Subject 2's unaffected older brother showed no evidence of fragile X sites in 50 cells examined. Subject 2's chromosome spreads were further studied using fluorescent in situ hybridization (FISH) with probe C10B52, which is known to map distal to *FRAXA* and proximal to *FRAXE*. This probe gave a signal proximal to the fragile site in 13 of 17 metaphases examined (data not shown, Baker and Sutherland, personal communication), consistent with the *FRAXE* site.

For subject 2's family, Southern blot assessment of the *FMR1* promoter region did not indicate the presence of an abnormal CGG expansion or of hypermethylation. For the proband, PCR analysis was used to confirm a normal-sized allele of 29 CGG repeats.

Of the members of subject 2's family tested for *FRAXE*, only the proband and his mother showed the GCC expansion characteristic of a mutation at that site. For subject 2, the GCC expansions found in peripheral lymphocyte cells were heterogeneous, resulting in a "smearing" of DNA fragments >5.2 kb (Fig. 1, lane 9). The expansion pattern for lymphoblastoid-derived cell lines was a discrete band at 7.5 kb (Fig. 2, lane 9). For subject 2's biological mother, the Southern blot pattern was one of two discrete bands corresponding to the normal X chromosome-derived allele at 5.2 kb and the *FRAXE* allele at approximately 6.2 kb. Both mother (data not shown) and son (Fig. 2, lane 12) carried expansion alleles which were refractory to digestion by *Not*I, and additional assays with subject 2's DNA showed his alleles were also completely refractory to digestion with *Bss*HII and *Sac*II (Fig. 2, lanes 10–11).

Medical and Developmental History

Subject 1. Subject 1's pre- and perinatal history were unremarkable. As an infant he experienced several episodes of otitis media which did not affect auditory functioning. Based on parental report, subject 1 first sat up at age 10 months, first walked at age 13 months, and first began to use words at age 1 year, 2 months. Physical and neurological examination conducted at ages 1 year and 1 year, 3 months detected mild-to-moderate hypotonia, and mild gross motor, and speech delays. As of age 4 years, 4 months, subject 1 was receiving speech therapy.

Subject 2. Subject 2's pre- and perinatal history were also unremarkable. His parents reported that he first sat up at age 9 months, first walked at age 1 year, 5 months, and began to use words at age 3 years, 6 months. Subject 2's parents initially became concerned about their son's behavior when he was age 2 years, 6 months, and by 4 years, 8 months, he was formally diagnosed with "variable cognitive profile," communication disorder, and "autistic-like features." Physical examination at age 4 years, 8 months was relatively unremarkable, including normal head circumference (50–75th centile), height, weight, and testicular size; however, he did demonstrate facial hypoplasia, "lopped ears," and an inter-pupillary distance at the 3rd centile. Excepting his serious learning and behavioral problems, subject 2 has not experienced any significant

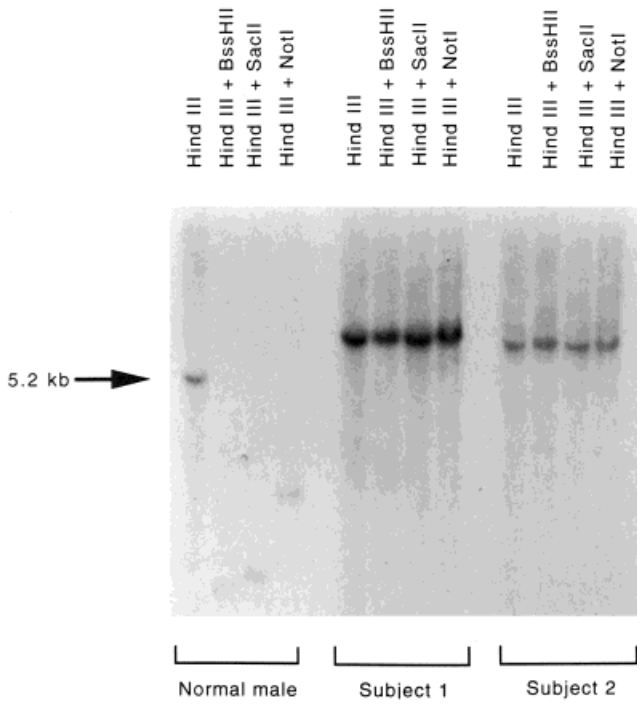

Fig. 2. Methylation analysis of the *FRAXE* locus in lymphoblastoid-derived DNA. Subclone OXE20 was used as a hybridization probe against a Southern blot containing DNA digested with *Hind*III and one other methylation-sensitive restriction enzyme (*Bss*HII, *Sac*II, or *Not*I). In the normal male, the *Hind*III 5.2-kb fragment is sensitive to digestion by these enzymes, resulting in smaller fragment sizes. In both subjects 1 and 2, the same *Hind*III fragment was refractory to digestion, indicating hypermethylation at the *FRAXE* CpG island. Band sizes for subjects 1 and 2 are 8.0 and 7.5 kb, respectively.

health difficulties. Since entering school he has been placed in special education classes designed mainly to cope with his language and behavioral problems.

Cognitive Evaluations

Subject 1. Select cognitive and behavioral evaluations for both subjects are summarized in Table I. At age 1 year, 5 months, subject 1 scored markedly below average on the Bayley Mental Developmental Index (MDI = 63) [Bayley, 1993], although his Vineland Adaptive Behavior Scale scores [Sparrow et al., 1984], which did not include the motor domain, were within normal limits for his age. At age 4 years, 4 months, subject 1's Stanford-Binet composite IQ [Thorndike et al., 1986] was low-average (IQ = 88). The area scores were relatively even, except for the abstract visual reasoning area, which was below average (Table I). This low score was primarily the result of subject 1's poor performance in copying an arrangement of three blocks modeled by the examiner. Subject 1's Vineland Adaptive Scale domain scores from age 4 years, 4 months were low-average except for the motor skills domain score, which was markedly below average (Table I). Other tests of visual-motor integration indicated that subject 1 scored below average on tasks requiring motor coordination (SS = 82), and average on those requiring only visual perception. Subject 1's below-average score on the Preschool Language Scale (SS = 82) reflected delayed expressive and receptive language ability.

Subject 1's mother. Subject 1's biological mother scored in the normal range on the WAIS-R (IQ = 97) [Wechsler, 1981] and on several neuropsychological assessments. Her WAIS-R profile was relatively consistent across subtests, with the weakest performance occurring on information, arithmetic, and similarities (scaled scores = 6).

Subject 2. Clinical records revealed that at age 4 years, 5 months, subject 2's Stanford-Binet IQ was 78. A follow-up assessment 5 months later noted that subject 2 had very poor language abilities and organizational skills, and above-average visual-spatial memory.

At age 8 years, 2 months, subject 2 received a Stanford-Binet composite IQ score of 88, with high-average skills in the quantitative area and average abstract visual reasoning scores (Table I). His verbal area score was just below average, and his short-term memory area score was markedly below average; however, these scores represent a minimal estimate because of subject 2's noncompliance and impulsivity during testing. Vineland Adaptive Behavior Scale scores from age 8 years, 2 months were markedly below average across domains (Table I).

At age 10 years, 5 months, in accordance with his county's special education procedures, subject 2 received an educational assessment which included the Stanford-Binet and the Vineland Adaptive Scales. His cognitive and adaptive profile at age 10 years, 5 months was very similar to that seen at age 8 years, 2 months, except for higher abstract visual and short-term memory area scores (Table I).

At age 12 years, 3 months, subject 2's WISC-R [Wechsler, 1974] full-scale IQ score was 100. On this assessment he demonstrated a distinct verbal performance split characterized by above-average visual-spatial scores, markedly below-average vocabulary (scaled score = 4) and comprehension (scaled score = 3) scores, and a low-average digit span score (scaled score = 7). Psychoeducational testing indicated above-average math achievement and average achievement in writing and reading. His Vineland socialization score was well below average (Table I). The neuropsychological testing battery did not indicate any exceptional scores, except for a high number of perseverative errors on the Wisconsin Card Sort Test, and a below-average score on semantic word fluency (data not presented). Subject 2 demonstrated normal hearing and articulation abilities and average ability to follow verbal directions, although he performed well below average on sentence formation tasks.

Subject 2's mother. Subject 2's biological mother scored in the normal range on the WAIS-R (IQ = 91) and on all neuropsychological assessments except for a notable weakness (8-year-old level) on the Hiskey-Nebraska spatial reasoning test [Hiskey, 1966]. Her WAIS-R subtest scores were even, except for a markedly below-average score on the picture completion subtest (scaled score = 4), a task which requires one to identify what is missing from a sketch of a concrete object (e.g., the handle missing from a suitcase).

Behavioral Evaluations

Subject 1. Subject 1 met DSM-III-R criteria for mild attention deficit hyperactivity disorder (ADHD) at age 4 years, 4 months, a diagnosis which cannot be reliably assessed at age 1 year, 5 months. Other mild-to-moderate behavior problems noted by subject 1's parents included body rocking at age 1 year, 5 months, and repetitive language and perseverative thoughts at age 4 years, 4 months. Subject 1 did not meet diagnostic criteria for autistic disorder or pervasive developmental disorder at either age.

Subject 1's mother. Subject 1's biological mother did not receive any standardized psychiatric evaluations. Her interactions with professionals related to the evaluations of her son did not indicate any obvious psychopathology.

Subject 2. Subject 2 met diagnostic criteria for autistic disorder and mild-to-moderate ADHD during both evaluations at ages 8 years, 2 months, and 12 years, 3 months. Mild-to-moderate problems with irritability and conduct were reported by his parents. Autistic-spectrum behaviors, irritability, and conduct problems were further documented in his educational reports.

Subject 2's mother. Based on information collected from several standardized interviews and self-administered questionnaires, no psychopathology was evident in subject 2's mother with three exceptions: 1) a single episode of major depression, 2) the diagnosis of social phobia reflecting her anxiety about test-taking and performing in front of others, and 3) an interpersonal sensitivity factor score on the Hopkins Symptom Checklist [Lipman et al., 1979] that was more than two standard deviations above the mean reported for a control group [Reiss et al., 1993].

Neuroanatomical Evaluations

Measured brain areas and volumes for each subject and corresponding comparison data are presented in Table II. The comparison data are from two male groups previously studied in our neuroimaging laboratory [Reiss et al., 1991, 1995]. One group was composed of individuals with normal IQ, and the other of individuals with the *FRAXA* mutation and mental retardation.

Subject 1. The only midsagittal-area measure which clearly distinguished subject 1 from normal controls was the posterior vermis (lobules VI–X) measure (Fig. 3), which was two standard deviations larger than that of the means reported for the control and *FRAXA* comparison groups (Table II). Compared to the *FRAXA* group, subject 1 also demonstrated a smaller fourth ventricular area. No volume measures differentiated subject 1 from normal IQ subjects. However, subject 1 did have a markedly smaller caudate nucleus compared to the *FRAXA* group mean.

Subject 2. The only midsagittal measure which distinguished subject 2 from the normal IQ controls was the fourth ventricular area, which was larger in subject 2. The *FRAXA* group had a mean fourth ventricular area, similar to that of subject 2. The only volume measure which distinguished subject 2 from *FRAXA* and normal IQ controls was the thalamic nucleus volume. Subject 2's thalamic nucleus was larger than the mean reported for either comparison group (Table II).

DISCUSSION

Comparison to Other *FRAXE* and *FRAXA* Males

Since the discovery of the *FRAXE* locus [Sutherland and Baker, 1992], most of what is currently known about the associated phenotype has been described in five papers [Knight et al., 1993, 1994, 1996; Hamel et al., 1994; Mulley et al., 1995]. These studies include descriptions of 36 males (adults and children) from 13 different families who had *FRAXE* GCC repeat lengths >130, a size above which the CpG island is likely to be hypermethylated [Hamel et al., 1994]. Nearly all of these cases (32/36) were of individuals with cognitive impairment ranging from learning problems and speech delay to mental retardation. In only 5 cases were standardized IQ scores reported. These scores ranged from 53–104 and demonstrated variable cognitive profiles [Hamel et al., 1994; Mulley et al., 1995; Knight et al., 1996]. Subjects 1 and 2 described in this report had higher IQ scores than those previously reported for most *FRAXE* males. Although subject 1's cognitive profile was relatively consistent across subtests, subject 2 demonstrated a clear math/language discrepancy. Therefore, it cannot be deduced from existing data that the *FRAXE* mutation is associated with a specific profile of cognitive deficits. General and variable cognitive deficits, however, may be related to the *FRAXE* genotype.

The 2 males described in this report demonstrated cognitive characteristics which distinguished them from the average male subject with the *FRAXA* mutation. For example, the *FRAXE* subjects had full-scale IQs in the normal range and cognitive profiles which did not show a discrepancy pattern typically seen in individuals with *FRAXA*. Most *FRAXA* males are moderately to severely mentally retarded, and demonstrate visual-spatial weaknesses and verbal/comprehension strengths [Freund and Reiss, 1995]. Furthermore, males with the *FRAXA* full mutation have been shown to experience a decline or plateau in IQ which occurs in early to late childhood [Hagerman et al., 1989; Dykens et al., 1993]. The IQ scores of the two *FRAXE* subjects presented in this report did not decline with age (Table I).

Both *FRAXE* subjects 1 and 2 were diagnosed with ADHD, suggesting that *FRAXE*, similar to *FRAXA*, may play a role in the etiology of mild-to-moderate inattention and overactivity. ADHD is considerably more common in males with *FRAXA* than in IQ-matched controls [Baumgardner et al., 1995]. Accordingly, if ADHD is a salient feature of *FRAXE* it may be one aspect of overlap with the *FRAXA* phenotype. Data from previous *FRAXE* studies, however, are less compelling. Problems with attention and overactivity were reported for only 2 cases [Hamel et al., 1994]. More data are therefore needed to confirm ADHD as a cognitive/behavioral correlate of the *FRAXE* mutation.

In the area of behavioral self-regulation and daily functioning, the Vineland Adaptive Behavior scores for subject 1 were consistent with his full-scale IQ. For subject 2, however, Vineland scores were significantly be-

TABLE II. Brain Measurements From *FRAXE* Subjects Compared to Published Means From Normal and *FRAXA* Controls

	Subject 1	Subject 2	Normal IQ controls[a]	*FRAXA*[a]
Age (years) for area measurements (units = cm^2)	4	12	Mean, 13; range, 1–32	Mean, 16; range, 2–43
Corpus callosum	5.64	6.87	6.81 ± 1.38	7.20 ± 1.45
Anterior vermis (I–V)	4.47	5.79	4.71 ± .58	4.83 ± .53
Posterior vermis (VI–X)	7.99	6.98	6.67 ± .64	5.70 ± .87
Midbrain	2.41	3.17	3.02 ± .50	2.88 ± .19
Pons	4.67	5.96	6.05 ± .83	6.09 ± .87
Fourth ventricle	0.78	1.65	.96 ± .25	1.47 ± .34
Age (years) for volume measures (units = cm^3)	4	12	12 ± 8	11 ± 8
Total cerebrum	1,180.6	1,482.7	1,291.2 ± 125.2	1,296.3 ± 98.9
Extraventricular CSF	56.4	127.5	84.7 ± 26.3	91.4 ± 18.6
Lateral ventricular CSF	5.62	10.91	14.8 ± 8.2	23.7 ± 11.0
Cortical gray matter	695.3	774.2	664.3 ± 76.0	677.4 ± 79.4
White matter	392.1	534.6	493.5 ± 92.8	466.5 ± 84.0
Subcortical gray	31.1	33.9	32.5 ± 4.4	36.6 ± 4.3
Caudate nucleus	9.5	11.00	11.1 ± 1.7	13.8 ± 1.5
Lenticular nucleus	9.3	7.0	9.5 ± 1.7	10.2 ± 1.9
Thalamic nucleus	12.3	15.9	11.9 ± 2.0	12.6 ± 1.6

[a] Means and standard deviations are from Reiss et al. [1991, 1995].

low his full-scale IQ (Table I). This adaptive skills/cognitive discrepancy differentiated subject 2 from subject 1, and from *FRAXA* and developmentally delayed controls [Baumgardner et al., 1995]. Subject 2's irritability factor score on the Parent Aberrant Behavioral Checklist [Freund and Reiss, 1991] was more than one standard deviation above the means from males with *FRAXA* and males with non-*FRAXA* developmental delay. Similarly, temper tantrums and/or aggressive behaviors were noted in 2 other males with the *FRAXE* mutation [Mulley et al., 1995; Knight et al., 1996]. These data point to irritability as a potential correlate to the *FRAXE* mutation worth further exploration.

Subject 2 was unequivocally diagnosed with autistic disorder, while subject 1 demonstrated limited autistic-spectrum behaviors (repetitive thoughts and language, and body rocking) which may have been related to developmental age. Autistic-spectrum behaviors, including rocking, impaired social interaction, hand-flapping, repetitive vocalizations, and body spinning, have been observed in at least 4 other *FRAXE* cases [Wang et al., 1993; Knight et al., 1994, 1996]. Additionally, autistic-spectrum motor stereotypies were noted in 2 other subjects with DNA deletions that included the *FRAXE* gene (*FMR2*) [Gedeon et al., 1995; Chakrabarti et al., 1996; Gecz et al., 1996].

FRAXA is known to be associated with a specific set of autistic behaviors including: a) dysfunction in peer play, but healthy attachment to parents, b) gaze aversion, c) unusual rate, rhythm, and tone to speech, d) lack of fantasy play, e) echolalia, and f) verbal perseveration [Reiss and Freund, 1990]. Subject 2 demonstrated all these traits, indicating partial similarity with the *FRAXA* autistic-spectrum phenotype. Subject 1 had only some mild echolalia and perseverative behaviors. An association between *FRAXE* and autistic-spectrum abnormalities is suggested by these data and should be investigated further. However, available data currently fall short of confirming an association between autism or even autistic-spectrum behaviors and *FRAXE*.

The quality of subject 2's autism is worth noting because it existed in the context of normal IQ with high-average math and exceptional graphic skills. It was learned during this subject's second evaluation at age 12 years, 3 months that he was able, from memory, to draw detailed and proportional maps of several of the continents. Criteria for Asperger syndrome did not apply in this case because of subject 2's significant language delay (DSM-IV) [American Psychiatric Association, 1994]; however, high-functioning autism of autistic savant status may be worth considering when evaluating other *FRAXE* children.

The neuroanatomy of the 2 *FRAXE* subjects presented do not show any consistent differences from controls, although these comparisons are limited by the broad age range of the control groups (Table I). The increased thalamic and fourth ventricular sizes in subject 2 are dramatic, but are isolated and must therefore be viewed with caution. Using the same measurements, a neuroanatomical phenotype was previously elucidated in males with the *FRAXA* mutation [Reiss et al., 1991, 1995]. This *FRAXA* phenotype includes enlarged ventricular and caudate nucleus volumes, and reduced cerebellar vermis area compared to controls. The *FRAXE* subjects, except for an enlarged fourth ventricle in subject 2, showed no such differences. In fact, subject 1 had a markedly small caudate and fourth ventricle, and a large vermis compared to the *FRAXA* group.

FRAXE Females

In addition to the 2 probands reported in this paper, 3 females from the two families identified were *FRAXE*-positive. At least 30 females (adults and children) with large and presumably methylated GCC expansions (> 130 repeats) have been mentioned in other publications [Knight et al., 1993, 1994; Hamel et al., 1994;

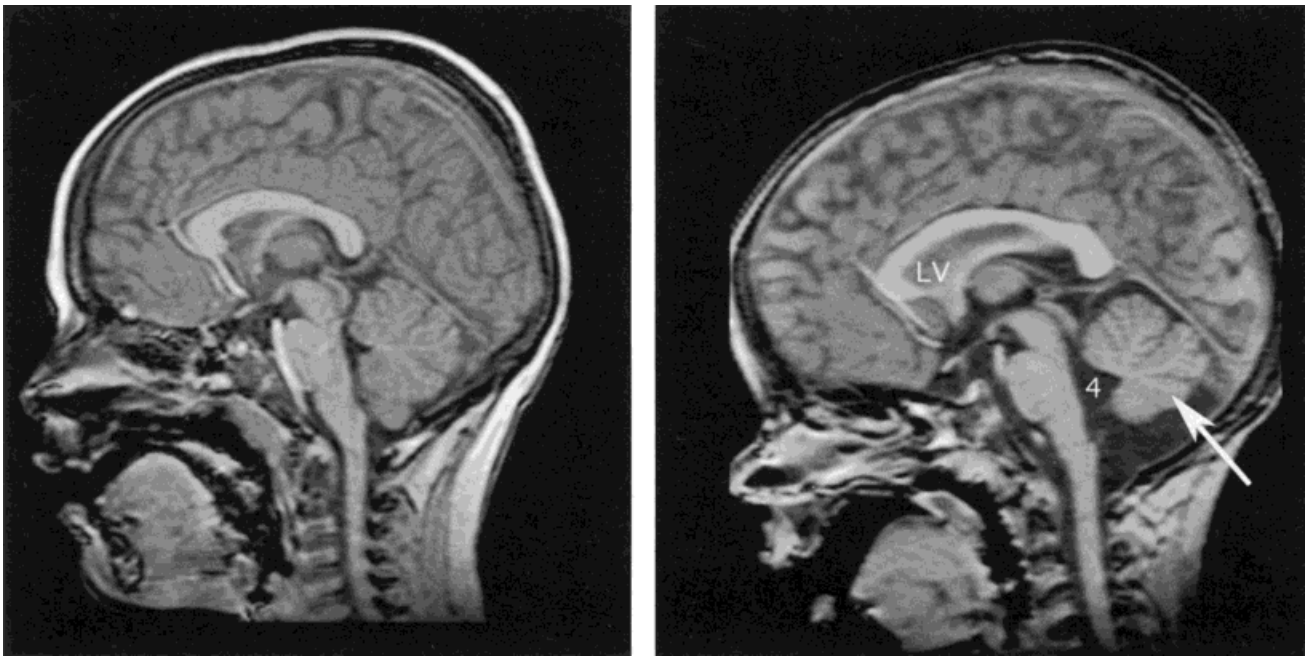

Fig. 3. Representative MRI scans. Left: Midsagittal view of *FRAXE* subject 1 (age, 4 years; IQ, 88). Right: Male with *FRAXA* (*FMR1*) mutation (age, 4 years; IQ, 78). Reduction in the posterior cerebellum vermis (arrow), and enlargement in the lateral ventricles (LV), are evident in the *FRAXA* child. These structural differences have previously been observed in studies comparing *FRAXA* subjects to developmentally delayed and normal controls [Reiss et al., 1991, 1995].

Mulley et al., 1995]. Of these 30 females, 20 demonstrated no obvious learning or behavioral problems, 8 were described as "impaired" or in a special school, and 2 were moderately mentally retarded, indicating an overall penetrance rate of 33%. Consistent with an X-linked trait, the expression of the *FRAXE* cognitive phenotype appears milder in females than in males.

Like the majority of such females described to date, the 3 females in this report had average cognitive ability. Subject 1's grandmother experienced no substantial learning or behavioral problems according to her daughter. Subject 1's mother had an IQ of 91 with a relatively flat profile across subtests. Subject 2's mother had an IQ of 97 with an even profile, except for notable difficulties on two specific subtests (Hiskey-Nebraska Spatial Reasoning and the WAIS-R Picture Completion), which may reflect a visual-spatial weakness. From previous studies, quantitative IQ data were reported for 3 female subjects with the *FRAXE* expansion. Two of these subjects had a borderline full-scale IQ (72–73) and one was mentally retarded (full-scale IQ = 49). None of these 3 subjects had subtest scores indicating a visual-spatial weakness or some other specific cognitive profile.

Behavioral evaluations of subject 2's mother revealed a history of major depression and considerable social anxiety. A single episode of major depression may be related to factors other than the *FRAXE* mutation, as a previous report indicates that females with developmentally delayed children often have some history of depression [Reiss et al., 1991]. Moreover, both depression and social anxiety are not uncommon in the general female population [Kaplan et al., 1994].

CONCLUSIONS

This is a report on 2 unrelated male individuals with the *FRAXE* GCC expansion mutation and corresponding hypermethylation at this X-chromosome locus. The cognitive and behavioral data presented on these males were partially suggestive of a *FRAXE* phenotype, and were in many ways inconsistent with the *FRAXA* phenotype. The neuroanatomical data further distinguished these *FRAXE* subjects from individuals with *FRAXA*.

In support of a specific *FRAXE* phenotype, there was some overlap between the characteristics of these 2 *FRAXE* subjects and other *FRAXE* subjects previously described. However, there were also some clear discrepancies which indicate that *FRAXE* may be a coincidental finding among some individuals ascertained for nonspecific developmental delay. This study and reports like it are limited by the current paucity of *FRAXE* cases [Allingham and Ray, 1995; Wang et al., 1995; Knight et al., 1996], and by the lack of standardized assessments used to evaluate these cases. Nevertheless, the *FRAXE* mutation does appear to be associated with nonspecific developmental delay at a frequency greater than that expected by chance [Mulley et al., 1995]. Moreover, it can be hypothesized from this report and others, that the *FRAXE* mutation may be associated with low-normal IQ to moderate mental retardation, communication deficits, attention problems, and overactivity. A possible association with autistic behavior is also suggested. Future work with larger samples and new *FRAXE* cases is essential to confirm these preliminary inferences.

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APPENDIX A. List of Assessments Not Mentioned in Text*

Subject 1 (administered at age 4 years, 4 months)
Preschool Language Scale-3
Developmental Test of Visual Motor Integration
Developmental Test of Visual Perception
Subject 2 (administered at age 12 years, 3 months)
Woodcock Johnson Tests of Achievement-R
Boston Naming Test
Rapid Automized Naming
Rey Osterich Complex Figure Drawing
Judgment of Line Orientation
Face Recognition
Test of Facial Affect Recognition
Prosody Content Congruity Task
Category Fluency (animals and foods)
Letter Word Fluency
Test of Language Development-2, Intermediate
Token Test for Children
Arizona Articulation Proficiency
Test of Auditory Discrimination

* For more details, contact the authors.

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